



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11) **EP 1 010 430 A1**

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
21.06.2000 Bulletin 2000/25

(21) Application number: **99917168.9**

(22) Date of filing: **27.04.1999**

(51) Int. Cl.⁷: **A61K 38/02, A61K 7/26,
A23G 3/30, A23J 3/08,
A23L 1/305**

(86) International application number:
PCT/JP99/02223

(87) International publication number:
WO 99/56762 (11.11.1999 Gazette 1999/45)

(84) Designated Contracting States:
CH DE ES FR GB IT LI NL

(30) Priority: **30.04.1998 JP 13424398**

(71) Applicant:
**SNOW BRAND MILK PRODUCTS, CO., LTD.
Sapporo-shi Hokkaido 065-0043 (JP)**

(72) Inventors:
• **TAKADA, Yukihiro**
Kawagoe-shi Saitama-ken 350-0811 (JP)
• **AOE, Seiichirou**
Sayama-shi Saitama-ken 350-1331 (JP)

• **SERIZAWA, Atsusi**
Arbein Kawagoe Fujimikoen
Kawagoe-shi Saitama-ken 350-1165 (JP)
• **SUGURI, Toshiaki**
Tokyo 116-0011 (JP)
• **DOUSAKO, Shunichi**
Urawa-shi Saitama-ken 336-0002 (JP)

(74) Representative:
VOSSIUS & PARTNER
Siebertstrasse 4
81675 München (DE)

(54) **PREVENTIVES AND AMELIORATING AGENTS FOR PERIODONTOSIS**

(57) Preventives and ameliorating agents for periodontosis which contain as the active ingredient a basic protein originating in milk which is obtained by bringing milk or a material originating in milk with a cation exchange resin and then eluting the adsorbed fraction with an eluent and has an isoelectric point of from 7.5 to 11; and foods, drinks, toothpastes or drugs such as throat washings containing these preventives and ameliorating agents.

EP 1 010 430 A1

DescriptionTechnical Field

- 5 [0001] This invention relates to a periodontal disease preventive and ameliorative agent with a milk-derived basic protein as an effective ingredient. This invention also relates to the utilization of the periodontal disease preventive and ameliorative agent with a milk-derived basic protein as an effective ingredient for a food/drink and medicament.

Background Art

10

[0002] In recent years, periodontal disease has become an object of public concern. Unlike dental caries, periodontal disease is a disease wherein the foundation of the teeth becomes weak and the use of teeth other than a decayed tooth is eventually lost. In addition, it is said that there are a large number of people who show symptoms of periodontal disease. In this sense, periodontal disease is a more serious disease than dental caries.

- 15 [0003] Conventionally, prevention of periodontal disease is conducted from a point of view to prevent bacteria, the cause of the disease, from propagating by removing dental plaque, or gargling with a mouthwash that contains antibacterial agent. But the effectiveness of these methods is considered to be low for advanced cases of periodontal disease. In other words, at the terminal stage of periodontal disease, a decrease in alveolar bone is observed and once alveolar bone is lost, it is difficult to reproduce it. Thus, if a tooth is lost due to periodontal disease, it interferes with people's daily life, because, needless to say, it is difficult to eat and it engenders pain, etc. Therefore, an immediate and effective preventive/ameliorative means is called for. However, at this moment no periodontal disease preventive and ameliorative agent, which is effective to curb decrease in alveolar bone, is available.
- 20

Disclosure of Invention

25

[0004] A problem that this invention is intended to solve is to provide a periodontal disease preventive and ameliorative agent with a milk-derived basic protein as an effective ingredient. Another problem that this invention is intended to solve is to provide a medicament and food/drink in which this periodontal disease preventive and ameliorative agent with a milk-derived basic protein as an effective ingredient is contained.

- 30 [0005] The inventors of this invention discovered that basic protein, which is contained in milk in a very small amount, has a remarkable effect on the prevention and amelioration of periodontal disease and came to complete this invention.

[0006] As ingredients, milk such as cow's milk, goat's milk and sheep's milk can be used. The above-mentioned different types of milk can be used as is, in the form of fresh milk, or in the form of prepared products such as powdered milk, skim milk, reduced milk, whey, etc.

35

[0007] The basic protein used in this invention is obtained by contacting the above-mentioned milk with cation-exchange resin, and then eluting a fraction adsorbed by the resin using an elution solution with a salt concentration of 0.1M~1.0M. Further, the basic protein obtained can be used after desalting or condensing it, if necessary.

- 40 [0008] Basic protein obtained in this way is a milk-derived basic protein fraction that has a molecular weight distribution of 3,000~80,000 Daltons by sodium dodecyl-polyacrylamide gel sulfate (SDS-PAGE) electrophoresis and has an isoelectric point of 7.5~11. Its main components are lactoferrin and lactoperoxidase and, as its amino acid constituent in protein, it contains basic amino acids by more than 15 weight percents.

[0009] In addition, the periodontal disease preventive and ameliorative agent of this invention has the above-mentioned basic protein as its main ingredient, and it can be blended into a food/drink and medicament, for example, in the form of toothpaste, gargling agents, candies, chewing gum, lozenges, etc. As a method of dosing as a medicament, it is desirable to apply about 1~50mg/day for an adult to the surface of teeth, divided into several applications.

45

[0010] Further, the basic protein of this invention is essentially the ingredient of milk and is considered to be safe. In fact, as the result of toxicity tests using rats, no acute toxicity was found.

50 Best Mode for Carrying Out the Invention

(Manufacturing example)

Preparation of milk-derived basic protein

55

[0011] After fully rinsing out a column in which 100g of cation-exchange resin (sulfonated chitoppearl manufactured by Fujibouseki) was placed using deionized water, unsterilized skim milk 101 was run through the column at a flow of 10 ml/min. After fully rinsing out the column with deionized water, 0.05M phosphate buffer (pH7) 21 containing 0.1M

sodium chloride was run through. Subsequently, gradient elution from 0.5M to 1.0M was performed using 0.05M phosphate buffer (pH7) containing 0.1M sodium chloride and 0.05M phosphate buffer (pH7) containing 1.0M sodium chloride to elute basic protein adsorbed by the resin and recover it. This eluate was desalted using a reverse osmosis membrane. After concentrating it, it was lyophilized, and powdered basic protein was obtained. By repeating this operation eight times and 821g of basic protein was obtained.

(Testing Example)

Evaluation of decreased alveolar bone:

[0012] 54 six-week-old golden hamsters were raised preliminarily for a week. Under etherization, sterilized silk suture No. 4 for surgical use was put 5 times around the tooth cervix part of M1 of the golden hamsters. They were raised by being fed food (D#2000; Keyes, P.H. and Jordan: Archs. Oral. Biol. 9: 377-400, 1964) to cause them to have periodontal disease. The hamsters were then divided into three equal groups of 18 each, the control group, the basic protein 0.5mg/ml group and the basic protein 5mg/ml group. Treatment to continuously soak the inside of oral cavity of the hamsters with basic protein of respective concentration for about 10 minutes twice a day was conducted. Three days, seven days and fourteen days after the start of this treatment, by selecting six hamsters from each group, after performing fixed perfusion using 2.5% glutaraldehyde solution (pH 7.4) for about 20 minutes, both sides of the lower jaw bone were removed and the amount of decreased alveolar bone was evaluated. After the removed jawbones were fixed with 2.5% glutaraldehyde solution, a soft X-ray photograph was taken. The photos were analyzed using an image analyzer (PIAS LA-555). By measuring an area between an enamel cement border and the alveolar bone top near M1, the amount of decreased alveolar bone was evaluated.

[0013] The results are shown in Table 1. The amount of decreased alveolar bone of the hamsters in the groups to which basic protein 0.5mg/ml and 5mg/ml were administered is significantly low and this effect was concentration-dependent. These results demonstrate that milk-derived basic protein inhibits decrease in the alveolar bone and it is effective for the prevention and the amelioration of periodontal disease.

[Table 1]

| The Effectiveness to Control the Decreased Alveolar Bone by Milk-derived Basic Protein | | | |
|--|------|---------------|---------------|
| Control | | Basic Protein | Basic Protein |
| | | 0.5mg/ml | 5mg/ml |
| Decreased Area (mm ²) | | | |
| 3 days after | 0.25 | 0.22 | 0.14 |
| 7 days | 1.21 | 0.83 ** | 0.36 ** |
| 14 days | 1.64 | 1.01 ** | 0.45 ** |

**Note: As contrasted with the control group, there are significant differences. (P<0.01)

(Example 1)

Manufacture of toothpaste for periodontal disease preventive and ameliorative purposes

[0014] Toothpaste for periodontal disease preventive and ameliorative purposes was manufactured by blending each ingredient with the percentage shown in Table 2 below and placing the product in a container.

[Table 2]

| | |
|-----------------|-----------------------|
| Glycerin | 70.0 (weight percent) |
| Silicon dioxide | 20.0 |
| Xanthan gum | 1.0 |

EP 1 010 430 A1

[Table 2] (continued)

| | |
|--------------------|-----|
| Mint flavor | 1.0 |
| Titanium oxide | 0.7 |
| Sodium fluoride | 0.3 |
| Distilled water | 6.5 |
| Milk basic protein | 0.5 |

(Example 2)

Manufacture of a gargle for periodontal disease preventive and ameliorative purposes

[0015] A gargle for periodontal disease preventive and ameliorative purposes was manufactured by blending each ingredient in the percentage shown in Table 3.

[Table 3]

| | |
|--------------------|----------------------|
| Ethanol | 8.0 (weight percent) |
| Perfume | 0.9 |
| Sorbitol | 5.0 |
| Propylene glycol | 5.0 |
| Milk basic protein | 0.1 |
| Distilled water | 81.0 |

(Example 3)

Manufacture of chewing gum for periodontal disease preventive and ameliorative purposes

[0016] According to the weight percentage shown in Table 4, after gum base was dissolved and each ingredient was blended in, the product was shaped, resulting in chewing gum for periodontal disease preventive and ameliorative purposes.

[Table 4]

| | |
|--------------------|-----------------------|
| Gum base | 20.0 (weight percent) |
| Corn syrup | 9.0 |
| Dextrose 1 hydrate | 10.0 |
| Lactose | 5.0 |
| Glycerin | 5.0 |
| Sugar | 50.0 |
| Milk basic protein | 1.0 |

(Example 4)

Manufacture of candies for periodontal disease preventive and ameliorative purposes ugar and glutinous starch syrup were blended at the weight percentages shown in Table 5. The mixture was boiled at 150°C (302°F) until it was condensed. After it was cooled to 115°C (239°F), milk basic protein in the amount of 0.1 weight percent was stirred in. After it was cooled on a cooling disk, it was shaped, resulting in candies for periodontal disease preventive and ameliorative purposes.

[0017]

[Table 5]

| | |
|------------------------|-----------------------|
| Sugar | 69.9 (weight percent) |
| Glutinous starch syrup | 30.0 |
| Milk basic protein | 0.1 |

Industrial Applicability

[0018] he periodontal disease preventive and ameliorative agent of this invention with milk-derived basic protein as its effective ingredient can inhibit alveolar bone decrease that occurs in the terminal stage of periodontal disease. Hence it greatly contributes to prevention and amelioration of this disease.

[0019] Also, since the periodontal disease preventive and ameliorative agent of this invention can be blended in a food/drink and medicament, for example, in the forms of toothpaste, gargling agents, candies, chewing gum, lozenges, etc. so as to effectively apply them to the surface of teeth, substantial preventive and ameliorative effects can be obtained.

Claims

1. A periodontal disease preventive and ameliorative agent comprising a milk-derived basic protein as its effective ingredient, which protein has an isoelectric point within a range of 7.5~11.
2. A periodontal disease preventive and ameliorative agent comprising a milk-derived basic protein as its effective ingredient, which protein is obtained by contacting a milk or milk-derived ingredient with cation-exchange resin, and then eluting a fraction adsorbed by the resin using an elution solution.
3. A food and drink for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein has an isoelectric point within a range of 7.5~11.
4. A food and drink for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein is obtained by contacting a milk or milk-derived ingredient with cation-exchange resin, and then eluting a fraction adsorbed by the resin using an elution adsorption solution.
5. A medicament for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein has an isoelectric point within a range of 7.5~11.
6. A medicament for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein is obtained by contacting a milk or milk-derived ingredient with cation-exchange resin, and then eluting a fraction adsorbed by the resin using an elution solution.
7. A toothpaste or gargling agent for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein has an isoelectric point within a range of 7.5~11.
8. A toothpaste or gargles for periodontal disease prevention and amelioration, comprising a milk-derived basic protein as its effective ingredient, which protein is obtained by contacting a milk or milk-derived ingredient with cation-exchange resin, and then eluting a fraction adsorbed by the resin using an elution solution.

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/JP99/02223

| A. CLASSIFICATION OF SUBJECT MATTER Int.Cl. ⁶ A61K38/02, A61K7/26, A23G3/30, A23J3/08, A23L1/305 According to International Patent Classification (IPC) or to both national classification and IPC | | |
|---|--|---|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int.Cl. ⁶ A61K38/02, A61K7/26, A23G3/30, A23J3/08, A23L1/305 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAPLUS (STN), REGISTRY (STN), MEDLINE (STN), WPIL (DIALOG) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | JP, 9-217693, A (Yoshihisa Naitou), 27 August, 1996 (27. 08. 96), | 1, 3, 5, 7 |
| Y | Claims ; Par. Nos. [0005], [0019], [0020] ; Examples & EP, 730868, A1 & CA, 2169810, A | 2, 4, 6, 8 |
| X | JP, 5-279266, A (K.K. Imuno Japan), 26 October, 1993 (26. 10. 93), | 1, 3 |
| Y | Claims ; Examples (Family: none) | 2, 4-8 |
| X | JP, 4-500368, A (Dana Dominique), 23 January, 1992 (23. 01. 92), | 1, 7 |
| Y | Claims & WO, 90/15591, A & EP, 429635, A & FR, 2648346, A & AU, 9059315, A | 2-6, 8 |
| X | JP, 1-501000, A (Otto M. Poulsen), 6 April, 1989 (06. 04. 89), | 1, 7 |
| Y | Claims & WO, 88/02600, A & EP, 293407, A & DK, 8605016, A & AU, 8781750, A & NO, 8802708, A & FI, 8802947, A | 2-6, 8 |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | |
| * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family | | |
| Date of the actual completion of the international search 23 June, 1999 (23. 06. 99) | | Date of mailing of the international search report 6 July, 1999 (06. 07. 99) |
| Name and mailing address of the ISA/ Japanese Patent Office | | Authorized officer |
| Facsimile No. | | Telephone No. |

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/02223

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|--------------------------|
| Y A | JP, 5-202098, A (Snow Brand Milk Products Co., Ltd.), 10 August, 1993 (10. 08. 93), Claims ; Examples & EP, 556083, A & AU, 9332059, A & NZ, 245789, A & US, 5516675, A | 2, 4, 6, 8 1, 3, 5, 7 |
| Y A | JP, 3-502921, A (Svenska Mejeriernas Riksforenings Ekonomi-AB), 4 July, 1991 (04. 07. 91), Claims ; Examples & WO, 89/04608, A & SE, 458818, B & AU, 8827180, A & FI, 9001562, A & NO, 9002328, A & DK, 9001245, A & EP, 390821, A & US, 5149647, A | 2, 4, 6, 8 1, 3, 5, 7 |
| X Y | Keiichi Fujita, et al., "Zengaku bassi o okonatta shiniku kounaienbyou no nirei", Journal of the Japan Veterinary Medical Association, 51(3), 1998 March, pp.157-162, Refer to the full text | 1 2-8 |
| X Y | Reeko Satou, et al., "Neko no kounaien ni taisuru lactoferrin ryouhou", Journal of the Veterinary Medicine, 47(12), 1994, pp.889-893, Refer to the full text | 1 2-8 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)